IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No: 2134
FOSTER *et al.*	Art Unit: 1654
Appl. No.: 09/937,484	Examiner: Audet, Maury A.
§ 371 Date: January 23, 2002	Atty. Docket: 1581.0870000/TJS/LDB
For: **Use of a Lectin or Conjugates for Modulation of C-Fibre Activity**	

Declaration Under 37 C.F.R. § 1.132 of Dr. John Chaddock

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, the undersigned, Dr. John Chaddock, do hereby solemnly and sincerely declare that:

1. I am a named inventor of the subject matter described and claimed in U.S. Patent Application No. 09/937,484.

2. I am currently Head of Molecular Biology at Syntaxin, Ltd. in the United Kingdom. As evidenced by the abridged version of my *curriculum vitae* (attached), I have been actively undertaking research in the field of lectin conjugates for the last 18 years. I am an expert in the field of modulating C-fibre neuron activity for therapeutic purposes.

3. I am familiar with the Office Action dated 29 November 2005 for the above-captioned case.

4. Lectin compounds are a well-recognized group of structurally and functionally related molecules that share common structural features. As a first structural feature, lectins possess a highly conserved binding site triad of amino acids, which is known as the "Asp-Gly-Asn triad". The conserved Asp-Gly-Asn triad is disclosed, for example, in Svensson *et al. J. Mol. Biol. 321*: 69-83 (2002) (enclosed herein as Exhibit A), and in Loris *et al. J. Mol. Biol. 335*: 1227-1240 (2004) (enclosed herein as Exhibit B).

5. As a second structural feature, the lectins possess a "lectin fold", which consists primarily of three β-sheets:

- a "flat" six-membered "back" β-sheet;
- a small "top" β-sheet; and
- a curved, seven-stranded "front" β-sheet.

The "lectin fold" is described in detail in Chandra *et al. Prot. Engin. 14*: 857-866 (2001) (enclosed herein as Exhibit C), and in Turton *et al. Glycobiology 14*: 923-929 (2004) (enclosed herein as Exhibit D). The "lectin fold" facilitates presentation of a substrate (sugar) to the lectin in a concave region of the "front" β-sheet. The highly conserved amino acid triad consisting of Asp, Asn and Gly is found within the "lectin fold."

6. The primary sequence of *Erythrina cristagalli* lectin (ECL) is disclosed in Figure 2 of Exhibit A. Alignment of the primary sequence of ECL with the sequence of the *Erythrina corallodendron* lectin (ECorL) in the same Figure 2 of Exhibit A shows that ECL shares 96% sequence homology with ECorL, and reveals 100% identity in the conserved Asp-Gly-Asn triad: Asp89, Gly107 and Asn 133.

7. The primary sequence of the first 244 amino acids of the *Erythrina corallodendron* lectin (ECorL) was first disclosed in Adar *et al. FEBS Lett. 257*: 81-85 (1989) (enclosed herein as Exhibit E). Comparison of the amino acid sequence of ECorL with that of other legume lectins

reveals a high degree of homology overall and 100% identity in the conserved Asp-Gly-Asn triad. *See* Figure 3 in Exhibit E. This highly conserved lectin binding site "Asp-Gly-Asn triad" is well known to those skilled in the art.

8. It is understood by researchers in the field of lectins that the Asp-Gly-Asn triad and the lectin fold are highly conserved distinguishing features of all lectins.

In this regard, Loris R. *et al., Proteins, 1994 Dec; 20(4):330-346* (enclosed herein as Exhibit F) describes monosaccharide recognition by two forms of lentil lectin, via a highly conserved triad of residues, namely Asp 81, Gly 99 and Asn 125. Thomas, C.J. and Surolia A., *Biochem. Biophys. Res. Commun 2000 Feb 16; 268* (enclosed herein as Exhibit G) confirms that an invariant triad of residues Asp 87, Gly 105 and Asn 137 coordinates recognition of L-fucose by fucose-binding legume lectins. These references confirm that the "Asp-Gly-Asn triad" is essential for ligand recognition in a variety of lectins whose crystal structures are known.

Rao VS. *et al., J. Biomol. Struct. Dyn 1998 Apr 15(5):853-860* (enclosed herein as Exhibit H) confirms, with reference to soybean agglutinin, that the invariant "Asp-Gly-Asn triad" is essential for binding carbohydrate. Together with an aromatic residue (Phe or Tyr), these three invariant residues provide the basic frame for the sugar to bind. This is confirmed by Rao VS. *et al., Int. J. Macromol. 1998 Nov; 23(4):295-307* (enclosed herein as Exhibit I), which reviews the sugar binding sites of Erythrina corallodendron (EcorL), peanut lectin (PNA), Lathyrus ochrus (LOLI) and pea lectin (PSL) and confirms that the invariant residue Asp (from loop A), the invariant residue Asn (from loop C) and the invariant residue Gly (from loop B) are required for a tight interaction with the sugar molecule.

8. I further state that all statements made on my own knowledge are true and that all statements made on information and belief are believed to be true and further that willful false

statements and the like are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the U.S. Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereupon.

Date: 11th April 2006

John Chaddock, Ph.D.

515090.1

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Chaddock, John Andrew	Head of Molecular Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Durham, UK	BSc.	1988	Molecular Biology / Biochemistry
University of Warwick, UK	Ph.D.	1992	Protein Biochemistry
Open University, UK	Cert. Mgmt	2003	Management

NOTE: The Biographical Sketch may not exceed four pages. Items A and B (together) may not exceed two of the four-page limit. Follow the formats and instructions on the attached sample.

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions and Employment

1992-1995 Postdoctoral Research, University of Warwick, UK
1995-1996 Postdoctoral Research, University of Warwick, UK
1996-2001 Scientist, Centre for Applied Microbiology & Research, Salisbury, UK
2002-2005 Senior Scientist, Health Protection Agency, Centre for Applied Microbiology & Research, Salisbury, UK
2005-2006 Head of Molecular Biology, Syntaxin Limited, Salisbury, UK

Other Experience

MSc Examiner in the field of protein toxin biochemistry
Co-supervisor of Ph.D. studentship at the University of Bath
Visiting lecturer at University of Bath

Professional Memberships

1988-present Member of Biochemical Society, UK
2002-2004 Member of International Association for the Study of Pain

B. Selected peer-reviewed publications (in chronological order).

1. Wales, R. Chaddock, J. A., Roberts, L. M. & Lord, J. M. (1992) Addition of an ER retention signal to the ricin A-chain increases the cytotoxicity of the holotoxin. *Exp. Cell Res.*, 203, 1-4.
2. Wales, R., Chaddock, J. A., Corben, E. B., Taylor, S. C., Roberts, L. M., Hartley, M. R. & Lord, J. M. (1993) Mutational analysis and possible applications of ribosome-inactivating proteins. In Beadle, D. J., Bishop, D. H. L., Copping, L. G., Dixon, G. K. & Hollomon, D. W. (eds.) BCPC Monograph No. 55: Opportunities for Molecular Biology in Crop Protection, pp99-111.
3. Chaddock, J. A. & Roberts, L. M. (1993) Mutagenesis and kinetic analysis of the active site Glu177 of ricin A-chain. *Protein Eng.*, 6, 425-431.
4. Chaddock, J. A., Lord, J. M., Hartley, M. R. & Roberts, L. M. (1994) Pokeweed antiviral protein (PAP) mutants which permit *E. coli* growth do not eliminate catalytic activity towards prokaryotic ribosomes. *Nucleic Acids Res.*, 203, 1536-1540.
5. Chaddock, J. A., Roberts, L. M., Jungnickel, B. & Lord, J. M. (1995) A hydrophobic region of ricin A-chain which may have a role in membrane translocation can function as an efficient non cleaved signal peptide. *Biochem. Biophys. Res. Commun.*, 217, 68-73.

6. Walker, D., Chaddock, A. M., Chaddock, J. A., Roberts, L. M., Lord, J. M. & Robinson, C. (1996) Ricin A-chain fused to a chloroplast-targeting signal is unfolded on the chloroplast surface prior to import across the envelope membrane. *J. Biol. Chem.*, 271, 4082-4085.
7. Chaddock, J. A., Monzingo, A. F., Robertus, J. D., Lord, J. M. & Roberts, L. M. (1996) Major structural differences between pokeweed antiviral protein and ricin A-chain do not account for their differing ribosome specificity. *Eur. J. Biochem.*, 235, 159-166.
8. Hartley, M. R., Chaddock, J. A. & Bonness, M. S. (1996) The structure and function of ribosome-inactivating proteins. *Trends Plant Sci.*, 1, 254-260.
9. Zhan, J., de Sousa, M., Chaddock, J. A., Roberts, L. M. & Lord, J. M. (1997) Restoration of lectin activity to a non-glycosylated ricin B chain mutant by the introduction of a novel N-glycosylation site. *FEBS Lett.*, 407, 271-274.
10. Chaddock, J. A., Purkiss, J. R., Friis, L. M., Broadbridge, J. D., Duggan, M. J., Fooks, S. J., Shone, C. C., Quinn, C. P & Foster, K. A. (2000) Inhibition of vesicular secretion in both neuronal and nonneuronal cells by a retargeted endopeptidase derivative of *Clostridium botulinum* neurotoxin type A. *Infect. Immun.*, 68, 2587-2593.
11. Chaddock, J. A., Purkiss, J. R., Duggan, M. J., Quinn, C. P., Shone, C. C. & Foster, K. A. (2000) A conjugate composed of nerve growth factor coupled to a non-toxic derivative of *Clostridium botulinum* neurotoxin type A can inhibit neurotransmitter release *in vitro*. *Growth Factors*, 18(2), 147-155.
12. Chaddock, J. A. and Melling, J. (2002) *Clostridium botulinum* and associated neurotoxins. Chapter 55. *Molecular Medical Microbiology*. Ed Max Sussman, Academic Press, London. 1141-1152.
13. Chaddock, JA., Herbert, MH., Ling, R, Alexander, FCG., Fooks, SJ., Revell, D, Quinn, CP., Shone, CC. & Foster, KA. (2002) Expression and purification of catalytically active, non-toxic derivatives of *Clostridium botulinum* toxin type A. Prot. Express. Purif., 25, 219-228
14. Turton, K., Chaddock, JA & Acharya, KR. Botulinum neurotoxins: structure, function and medicine. TiBS 2002, 27(11), 552-558.
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17. Chaddock, JA, Duggan, MJ, Quinn, CP, Purkiss, JR, Alexander, FCG, Doward, S, Fooks, SJ., Friis, L, Hall, Y, Kirby, ER, Leeds, NJ, Moulsdale, HJ, Dickenson, A, Green, GM., Rahman, W., Suzuki, Rie, Shone, CC and Foster, KA. Retargeted clostridial endopeptidases: Inhibition of nociceptive neurotransmitter release *in vitro*, and antinociceptive activity in *in vivo* models of pain. Movement Disorders 2004, 19(S8), S42-S47.
18. Hall, YHJ, Chaddock, JA, Moulsdale, HJ, Kirby, ER, Alexander, FCG, Marks, JD and Foster, KA. Use of a new in vitro botulinum neurotoxin antibody detection assay to assess novel vaccine candidates. J. Immunological. Methods 2004, 288 (1-2), 55-60
19. Turton, K., Natesh, R., Thiyagarajan, N., Chaddock, JA & Acharya, KR. 2004, Crystal structure of Erythrina cristagalli lectin with bound N-linked oligosaccharide and lactose. Accepted by Glycobiology
20. Sutton, JM, Wayne, J, Scott-Tucker, A, O'Brien, SM, Marks, PMH, Alexander, FCG, Shone, CC and Chaddock, JA. Preparation of specifically activatable endopeptidase derivatives of Clostridium botulinum toxins type A, B and C and their applications. Accepted by Protein Expression & Purification
21. Chaddock, JA & Marks, PMH. Clostridial neurotoxins: structure-function led design of new therapeutics. Cellular and Molecular Life Sciences, 2006, 63, 540-551.

Exhibit A
Appl. No. 09/937,484

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JMB

High-resolution Crystal Structures of *Erythrina cristagalli* Lectin in Complex with Lactose and 2′-α-L-Fucosyllactose and Correlation with Thermodynamic Binding Data

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The primary sequence of *Erythrina cristagalli* lectin (ECL) was mapped by mass spectrometry, and the crystal structures of the lectin in complex with lactose and 2′-α-L-fucosyllactose were determined at 1.6 Å and 1.7 Å resolution, respectively. The two complexes were compared with the crystal structure of the closely related *Erythrina corallodendron* lectin (ECorL) in complex with lactose, with the crystal structure of the *Ulex europaeus* lectin II in complex with 2′-α-L-fucosyllactose, and with two modeled complexes of ECorL with 2′-α-L-fucosyl-*N*-acetyllactosamine. The molecular models are very similar to the crystal structure of ECL in complex with 2′-α-L-fucosyllactose with respect to the overall mode of binding, with the L-fucose fitting snugly into the cavity surrounded by Tyr106, Tyr108, Trp135 and Pro134 adjoining the primary combining site of the lectin. Marked differences were however noted between the models and the experimental structure in the network of hydrogen bonds and hydrophobic interactions holding the L-fucose in the combining site of the lectin, pointing to limitations of the modeling approach. In addition to the structural characterization of the ECL complexes, an effort was undertaken to correlate the structural data with thermodynamic data obtained from microcalorimetry, revealing the importance of the water network in the lectin combining site for carbohydrate binding.

Keywords: crystal structure; glycobiology; lectin; protein–carbohydrate interactions; structure/function

Introduction

Specific recognition of carbohydrates by proteins lies at the heart of many biological processes, ranging from cell–cell interaction and adhesion of infectious agents to host cells, cellular signaling and differentiation, malignancy and metastasis, to fertilization and immune response.[1,2] Understanding the roles of carbohydrates in these processes and how they interact with proteins is expected to have a large impact on the development of new treatments against many human diseases.

Legume lectins are especially well suited as a model system to study the molecular basis of protein–carbohydrate recognition because they are structurally similar and yet their specificity is diverse.[3–5] By now, the three-dimensional structures of 20 legume lectins have been solved by high-resolution X-ray crystallography, both in free form and in complex with a variety of carbohydrate ligands†.

Abbreviations used: ECL, *Erythrina cristagalli* lectin; ECorL, *Erythrina corallodendron* lectin; ELLA, enzyme-linked lectin assay; Fuc, L-fucose; fucosyllactose, FucLac, 2′-α-L-fucosyllactose; fucosyl-*N*-acetyllactosamine, 2′-α-L-fucosyl-*N*-acetyllactosamine; Gal, galactose; Glc, glucose; ITC, isothermal titration calorimetry; Lac, lactose; MPD, 2-methyl-2,4-pentanediol; MS, mass spectrometry; rms, root mean square; PBS, phosphate-buffered saline; UEA-II, *Ulex europaeus* lectin II, WBA-I and II, winged bean acidic lectin I and II.

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† See 3D Lectin Data Bank on World Wide Web URL: http://www.cermav.cnrs.fr/databank/lectine